THE ROLE OF PAP SMEAR IN DETECTION OF CYTOLOGICAL ABNORMALITIES OF UTERINE CERVIX IN HIV INFECTED WOMEN

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CERTIFICATE

This is to certify that the dissertation entitled **"THE ROLE OF PAP SMEAR IN DETECTION OF CYTOLOGICAL ABNORMALITIES OF UTERINE CERVIX IN HIV INFECTED WOMEN"** is a record of bonafide work done by Dr. M.Kavitha , Post graduate student in the Department of Pathology, Coimbatore under the supervision of **Dr. M.MURTHY**, Professor and Head, Department of Pathology, Coimbatore Medical college and submitted in partial fulfillment of the regulations of the Tamilnadu Dr. M.G.R Medical University, towards the award of M.D.Degree (Branch III) in Pathology.

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AIM OF THE STUDY

The present study is undertaken:

1) To study the role of Pap smear in HIV positive women attending Coimbatore Medical College Hospital.

2) To detect opportunistic infections, cervical dysplasia and cancer cervix in HIV positive women.

3) To increase the awareness of the value of Pap smear as an integral part of preventive care for HIV infected women.

INTRODUCTION

HIV/AIDS pandemic is emerging as one of the most serious health problems of this century and the focus is shifting fast from developed nations to developing countries including India due to its vast population[1].

In India, around 22% of total reported AIDS cases and 30% of newer HIV infections are among women[2]. HIV infection results in progressive depletion of immune system making the host susceptible to various opportunistic infections and other AIDS related malignancies.

Though the process cannot be stopped, it can be slowed down, which would improve the quality of life.

Vulvo vaginal candidiasis , Cervical dysplasia ,Cervical cancer in situ and Pelvic inflammatory disease(PID) were added to the revised classification system of HIV infection under category B[3].

Also cervical cancer has an increased occurrence and aggressiveness in HIV infected women. In 1993, The Center for Disease Control and Prevention (CDC) added Cervical Cancer to the list of AIDS defining malignancies [4].

It is very important for the HIV - positive women to have regular Pap smear. Pap smear is one of the important screening procedures which

was introduced by Dr George Papanicolaou and Traut in 1943 to lower the morbidity and mortality of cervical cancer by its early detection[5]. An abnormal pap smear can indicate inflammation, infection, dysplasia or cancer[6].

HIV infected women are 10 times more likely to have abnormal pap smears than HIV negative women[7]. Hence CDC recommends, all HIV positive women should have a complete gynecological examination, including a pap smear, when they are first diagnosed or when they first seek prenatal care[8] .Unlike most other cancers, Carcinoma Cervix is highly preventable when precursor lesions are detected and treated before they develop into cancer[9].

This study is undertaken to mainly highlight the importance of cervical cytology in HIV positive women and to increase the awareness about the value of Pap smear as an integral part of preventive care for HIV infected women.

REVIEW OF LITERATURE

India has a population of one billion, around half of whom are adults in the sexually active age group. The first AIDS case in INDIA was detected in 1986 and since then HIV infection has been reported in all states and union territories[10].

The spread of HIV in India has been uneven. HIV epidemics are more severe in the southern half of the country and the far north-east. The highest HIV prevalence rates are found in Andhra Pradesh, Maharashtra, Tamil Nadu and Karnataka in the south; and Manipur and Nagaland in the north-east[11].

In the southern states, HIV is primarily spread through heterosexual contact. Infections in the north-east are mainly found amongst injecting drug users and sex workers[12].

Estimated number of people living with HIV/AIDS, 2007[13]

People living with HIV/AIDS	2.31 million
Adult (15 years or above) HIV prevalence	0.34%

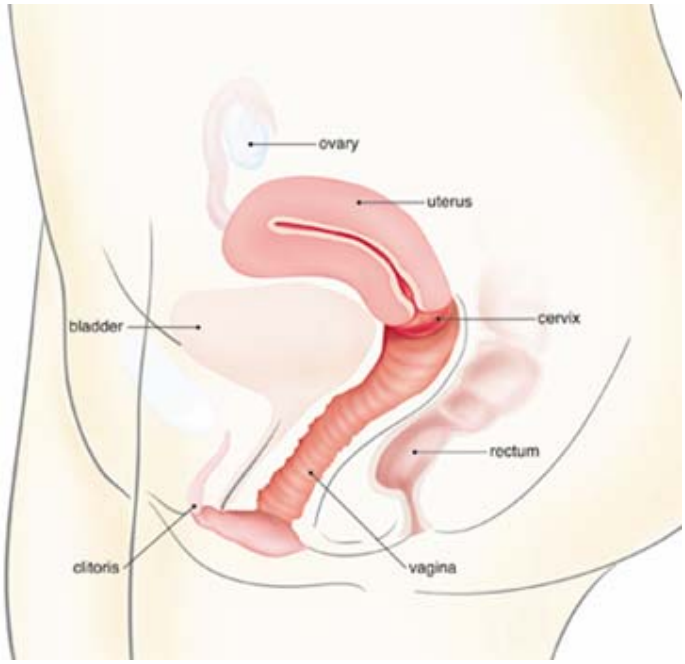
It is now thought that around 2.3 million people in India are living with HIV. Of these, an estimated 39% are female and 3.5% are children.

Women and the Biology of HIV Transmission

The majority of HIV infections are transmitted sexually and so most of the transmission occurs through the genital or reproductive systems of men and women[14].

The female genital tract

The female genital tract includes the vagina, cervix, uterus, fallopian tubes and ovaries.



Most HIV transmission in women happens through the vagina, the cervix and, possibly, the uterus.

The risk of transmitting HIV from men to women is much higher than from women to men. This is in part because of the much larger surface area of the

vagina and cervix .Women are exposed to considerable amounts of seminal fluid during sex, if ejaculation occurs[15].

The vagina is particularly vulnerable to invasion by bacteria, viruses and other germs. It is an ideal place for bacteria to grow, as it is warm and moist. It also provides an easy entry into the body.

Women with low levels of the hormone estrogen may be at increased risk for transmission of HIV because low estrogen levels directly affect the vaginal wall, making it thinner and accessible for easy passage of HIV through the wall[16].

Routes of HIV entry through the female genital tract: [17]

The vagina has various defense mechanisms against infection. The walls of the vagina are made up of mucous membrane that is thicker than the mucous membrane in other places where HIV transmission often happens, such as the rectum or cervix .The walls of the vagina have ten to twelve overlapping layers of epithelial cells, which create a strong barrier against germs such as viruses and bacteria. The vagina is also a home for Lactobacilli which are thought to give some protection against other bacteria and viruses that might infect the body.

It was thought that HIV can only get through the walls of the vagina through small tears or sores in the mucous membrane. Recent research suggests that HIV can pass between or through healthy cells. This means that HIV can still infect women even if the vaginal mucous membranes are healthy and intact.

Unlike the vagina, the mucous membranes lining the cervix and uterine walls have only a very thin layer of cells and so it is much easier for viruses like HIV to cross into the body through the cervix and possibly the uterus.

Because the cervix acts as a barrier to protect, it is a home for a large number of immune cells. Many of these immune cells are CD4+ cells, which are the most vulnerable to HIV.

Factors altering woman's susceptibility to HIV:

Hormonal changes:

Studies have suggested that the lining of the vagina gets thinner closer to menstruation than during other periods of a woman's cycle. This suggests that the risk of HIV infection over the course of menstrual cycle may vary.

Bacterial vaginosis:

Changes in the vaginal bacterial flora have been found to increase a woman's risk of HIV infection by as much as 2.5 times. This alteration in the vaginal

bacteria is referred to as altered vaginal flora (AVF) or bacterial vaginosis (BV) [18].

Age: Young women, particularly below the age of 24, appear to be much more vulnerable to HIV. This is because their genital tracts are not mature and may be more prone to tears and abrasions during sexual intercourse. Women who have gone through menopause are also at a higher risk of HIV infection, because the lining of the uterus becomes thinner and the vagina becomes drier.

Immature cervix (cervical ectopy): Up until the age of 18, a woman's cervix is still developing. During this time, the thinner cells that line the cervix are found further down into the vagina than they are in older women. This is called "cervical ectopy" or an "immature cervix". Since the cells lining the cervix provide a thinner and weaker barrier to HIV, young women with cervical ectopy have a much greater risk of HIV infection.

Pregnancy: Some researchers have found that pregnant women may be more at risk for HIV because of immunological and hormonal changes.

Sexually transmitted infections and HIV: [19]

Women are at more risk for sexually transmitted infections (STIs) than men. In addition, women often have fewer obvious symptoms, and therefore do not get

treatment until the infection has been present for a long time. Having an STI increases the risk of HIV transmission in several ways.

All STIs cause inflammation of the mucous membrane. Inflammation is the body's immune response to an infection or irritation. When the mucous membrane is inflamed, a large number of immune cells are recruited to fight the infection.

Many of those immune cells will be CD4+ cells or other immune cells that are involved in HIV transmission. In addition, when cells are fighting off an infection, they become activated. Activated CD4+ cells are more easily infected by HIV. Some STIs also cause open lesions or sores, which offer an easy way for the virus to get into the body and cause an infection.

Gynecological problems in HIV:

Gynecologic problems are common among HIV-positive women and are frequently present at the time of initial presentation for evaluation and care.

Minkoff et al. found that 46.9% of 262 HIV-infected women had atleast one incident gynecologic condition with serial assessment (Minkoff, 1999)[20]. In a study of women admitted to an inpatient AIDS service, although only 9% were admitted with a primary gynecologic problem, 83% had coexisting gynecologic disease when evaluated (Frankel, 1997)[21].

Some gynecologic issues are unrelated to the patient's serologic status, whereas others are directly related to HIV disease and associated immune suppression. Still others are associated epidemiologically with HIV because of common risk factors, such as sexual behavior or substance abuse.

HIV infection primarily affects women during their reproductive years, gynecologic and reproductive health care will play an increasingly important role in the overall care of the HIV-infected woman. With improved longevity and quality of life, gynecologic problems may be encountered more commonly or may be more prominent.

The Center for Disease Control and Prevention (CDC) added invasive cervical cancer to its list of AIDS-defining conditions in 1993. Still, much remains unknown about the incidence and natural history of this disease in HIV-positive women.

Pap smear screening of HIV positive women has demonstrated a four- to tenfold elevation in abnormal results compared to HIV negative women.

It is hoped that such screening appreciably decreases the potential for cervical cancer in HIV positive women.

Over the past ten years, a great deal of research has amassed regarding Pap smear findings and their management in HIV positive women.

In the setting of HIV infection 30–60% of Pap smears exhibit cytologic abnormalities and 15–40% have evidence of dysplasia; these rates are 10–11 times greater than those observed among HIV negative women (Maiman,1998)[22].

Malignancy in cervix is believed to begin to develop in adolescents or young adults and progress slowly over the decades. (Winkelstein,1990; Feldman, 1997).

HIV AND CERVICAL DYSPLASIA

Abnormal cervical cytology is more common among HIV-infected women and is associated with the presence of HPV infection and the degree of immune suppression.

Both frequency and severity of abnormal Pap smears and histologically documented dysplasia increase with declining CD4 counts and have also been associated with higher HIV-RNA levels (Garzetti, 1995; Shah, 1996; Davis, 2001)[23,24,25]

Incidence of abnormal Pap smears is increased in HIV-infected as compared to - uninfected women, and is associated with lower CD4 counts; progression and regression of Pap smear abnormalities have been associated with level of

immune suppression and plasma viremia as reflected in CD4 count and HIV viral load (Massad, 2001; Schuman, 2003)[26,27]

Increased HPV viral load, seen in women with more advanced HIV, is also associated with increased frequency, severity, and incidence of cervical dysplasia (Heard, 2000;Weissenborn 2003; Cohn, 2001)[28,29,30]

In HIV-positive women dysplasia is associated with more extensive cervical involvement and is more likely to involve other sites in the lower genital tract, such as the vagina, vulva, and perianal region (Hillemanns, 1996; Korn, 1995; Maiman, 1990; Petry, 1996; Williams, 1994)[31,32,33,34,35] as compared with HIV-negative women.

Recent studies have shown increased incidence of oncogenic HPV types (Minkoff, 1998) and increased incidence of biopsy-proven cervical dysplasia (Ellerbrock, 2000)[36] in HIV-positive women compared with HIV-negative controls.

A French study found an increased likelihood of progression of cervical abnormalities in HIV-positive women, although analysis was based on Pap Smear results only (Six, 1998)[37]

A recent study also found an association between progression of abnormal Pap smears and high plasma HIV RNA levels (>100,000 c/ml) (Sewell,

2000)[38].Presently there is little evidence for increased rates of progression to invasive cancer, particularly if adequate screening and treatment programs are in place.

INVASIVE CERVICAL CANCER IN HIV DISEASE:

In 1990, Maiman and colleagues evaluated 37 women diagnosed with invasive cervical cancer. Nineteen percent of the cancer patients under age 50 were HIV positive.

HIV positive patients had more advanced invasive disease than negative ones, and disease persisted or recurred in all positive patients compared to 37% of negative patients. Other researchers(Relliman, 1990; Schwartz, 1991; Maiman, 1993) [39,40] have published case reports of rapidly progressive invasive cervical cancer in women with HIV.

Maiman and his colleagues have continued to study the relationship between HIV and cervical cancer in a large cohort of women in Brooklyn, where there is a high female HIV rate. In this cohort, women with HIV infection and cervical cancer have a higher recurrence rate than HIV-negative women diagnosed at comparable stages of cancer (Maiman,1993). The HIV positive women had increased rates of severe dysplasia, larger lesions, and greater "field effect" than the women testing negative (Fruchte , 1997).

In a retrospective review of data on cervical cancer and AIDS in women registered through the New York City Department of Health and Institutional Tumor Registries from 1987 to 1995, Maiman (1997) found a strong correlation between cervical cancer and HIV disease throughout New York City.

The Gynecologic Oncology Group has recently begun a nationwide study (GOG 154) examining the issue of HIV testing and follow up for women younger than 50 with invasive cervical cancer. It is hoped that this will provide some insight concerning various clinical, pathologic, epidemiologic and demographic factors.

Oncogenic HPV types play a central role in the relationship between HIV and cervical cancer.

Biomedical research into the interaction between HIV and HPV in laboratory cell lines also was described (Cage, 1997). In the lab, HPV infected cells display an increased growth response when exposed to interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and the HIV-produced tat protein. Since HIV can up-regulate the production of IL-6 and TNF-α, there are several possible pathways by which HIV in the genital tract may enhance the growth potential for HPV infected cells, conceivably leading to an increased risk of cervical cancer.

Recent African data found that without high-risk HPV present, the risk ratio for cervical cancer between HIV-positive and HIV-negative women was approximately 1 (Hawes, 2003)[41].

Although there is little evidence that HIV infection is having a large effect on cervical cancer rates, linking of US AIDS and cancer registries has found that observed cervical cancer cases in HIV-infected women are up to 9-fold higher than the expected number of cases; however, the likelihood of cervical cancer was not related to CD4 count (Mbulaiteye , 2003)[42].

In an analysis of women in the HER study, the rate of invasive cervical cancer was 1.20/1000 person-years in HIV-infected women as compared to 0/1000 person-years in high-risk HIV-negative women (Phelps, 2001)[43].

Mean CD4 cell count was 443 cells/ mm3 at time of diagnosis of cervical cancer in women with HIV. Women with HIV and cervical cancer tend to be younger and less immune suppressed compared with HIV-positive women with other AIDS-defining conditions. Women with HIV and cervical cancer tend to be younger than HIV-negative women with cervical cancer (Lomalisa , 2000)[44].

A prospective cohort study from Italy found that the incidence of invasive cervical cancer as a first AIDS-defining condition continued to increase after the introduction of HAART, possibly due to the decrease seen in incidence of other AIDS-defining diseases after HAART (Dorrucci, 2001)[45].

HIV-positive women with invasive cervical cancer appear to present at more advanced stages (especially with CD4 < 200/ mm3), may metastasize to unusual locations , have poorer responses to standard therapy, and have higher recurrences and death rates, as well as shorter intervals to recurrence or death, compared with HIV-negative women of similar stage (Klevens, 1996; Maiman, 1990)[46].

Screening methods:

1. Cervical exfoliative cytology[47] (conventional Pap smear):

Exfoliative cervico vaginal cytology has been regarded as the gold standard for cervical screening programs. Pap smear is widely recognized as the most cost effective cancer screening test devised and serves as a model for screening of other malignancies. Pap smear is one of the important screening procedures which was introduced by Dr George Papanicolaou and Traut in 1943 to lower the morbidity and mortality of cervical cancer by its early detection.

Due to mass screening tests by Pap smear, the incidence of invasive carcinoma is grossly reduced. The availability of cervical cytology to screen for cervical cancer often permits diagnosis at the pre invasive stage, when treatment can almost always prevent progression to invasive cancer. For this reason, screening for cervical cancer is important in all women.

Screening for cervical cancer is of particular concern to HIV infected women and adolescents since the incidence of cervical intraepithelial neoplasia (CIN) and cancer cervix are four to five times higher in HIV-positive women and adolescents compared to HIV-negative women and adolescents with high risk sexual behavior .In the setting of HIV infection , 30–60% of Pap smears exhibit cytologic abnormalities and 15–40% have evidence of dysplasia; these rates are 10–11 times greater than those observed among HIV-negative women (Maiman,1998).

2) Schiller's iodine[48]:

In 1929 Schiller described this method. The normal cervical and vaginal epithelium rich in glycogen take up dark brown stain with iodine. Rapidly proliferating cells utilize all the glycogen and hence are deficient in glycogen. They remain unstained. Hence, iodine negative areas are considered as abnormal.

3) Visual inspection of the cervix with acetic acid[48] (VIA):

The technique is very simple and consists of examination of the cervix two minutes after 4% acetic acid application .Lesions which are stained acetowhite are regarded as positive.

4) Speculoscopy [48]:

Speculoscopy involves inspection of cervix following the application of 5% acetic acid with chemiluminiscent light and a low power magnification.

5) Cervicography [48]:

Cervicography involves taking photographs of the cervix using a special camera following the application of 5% acetic acid during a routine pelvic examination and pap smear collection. The photographs are then developed and the slide is projected on a 2×2 meter screen and read by an expert in colposcopy.

6) Colposcopic examination and biopsy [49]:

Colposcope was invented by Hinselman in 1954. Colposcope is inspection of lower genital tract under relatively low stereoscopic magnification and bright illumination. Topography, surface contour, vascular pattern, colour of cervix are all observed. Colposcopy is used to determine the exact distribution and extent of lesion, to select the site for direct biopsy, to confirm the cytological findings and to rule out invasive carcinoma.

RECOMMENDATIONS FOR PAP SMEAR SCREENING

US Public Health Service (USPHS)/Infectious Diseases Society of America (IDSA) 2001

HIV-infected women should have a complete gynecologic evaluation, including a pap smear and pelvic exam, as part of their initial evaluation. A Pap smear should be obtained twice in the first year after diagnosis of HIV infection. If these results are normal, annual examinations are then indicated.

More frequent Pap smears should be considered:

- with previous abnormal pap smear
- with HPV infection
- after treatment for cervical dysplasia in women with symptomatic HIV infection (including CD4 counts <200/mm3

Table: Pap Smear Report for Bethesda System[50]

Specimen adequacy	• Satisfactory for evaluation (note presence/absence of endocervical transformation zone component) • Unsatisfactory for evaluation (specify reason)
General categorization	• Negative for intraepithelial lesion or malignancy • Epithelial cell abnormality • Other

Interpretation/result	• Negative for intraepithelial lesion or malignancy
	  o infections
	  o reactive changes (inflammation, radiation)
	  o atrophy
	• Epithelial cell abnormalities
	  o atypical squamous cells (ASC)
	  ▪ of undetermined significance (ASC-US)
	  ▪ cannot exclude HSIL (ASC-H)
	  o low-grade squamous intraepithelial lesion, including HPV changes and mild dysplasia CIN1
	  o high-grade squamous intraepithelial lesion, including moderate and severe dysplasia, CIN2, CIN3
	  o squamous cell carcinoma
	  o glandular cell abnormalities
	• Other
	  o endometrial cells in a woman greater than or equal to 40 years of age

Table : Recommended Management for Abnormal Pap Smears

PAP SMEAR RESULT	MANAGEMENT
Unsatisfactory	Repeat Pap smear
Partially obscuring-inflammation	Evaluate for infection; consider repeat Pap smear
Epithelial cell abnormality	
- atypical glandular cells	Colposcopy, endocervical sampling; endometrial sampling if > 35 yrs. or with abnormal bleeding; cervical conization if initial evaluation negative and cytology favors neoplasia
- atypical squamous cells (ASCUS and ASC-H)	Colposcopy, biopsy if indicated; endocervical sampling if unsatisfactory colposcopy; follow with Pap every 6 months, consider repeat colposcopy annually if Pap unchanged
- low-grade squamous intraepithelial lesion (LSIL, CIN1)	Colposcopy, biopsy if indicated; endocervical sampling if unsatisfactory colposcopy; follow with Pap every 6 months, consider repeat colposcopy annually if Pap unchanged
- high-grade squamous intraepithelial lesion (HSIL, CIN2-3, carcinoma-in-situ)	Colposcopy, biopsy, endocervical sampling; treat with loop excision or conization
- invasive carcinoma	Colposcopy with biopsy or conization; treat confirmed invasive disease with surgery or

	radiation (referral to gynecologic oncologist needed)

HIV positive women should continue to be followed closely for evidence of lower genital tract neoplasia, regardless of antiretroviral therapy or viral load.

The goal of Pap smear screening is to prevent cervical and/or lower genital tract cancer. The Pap smear is a screening test with an average sensitivity of about 85% and an average specificity of about 98% .It is evident that the sensitivity and specificity of the Pap smear do not differ significantly among HIV-positive and HIV-negative women. Yet the proportion of HIV-positive women who receive abnormal Pap smear results is much higher than for HIV-negative women.

Management of Pap smear findings should rightly be a part of primary care of all women. This is based on a thorough review of the available research and clinical recommendations and a belief that Pap smear management is not outside the scope of primary care for HIV –positive women. Pap smear is a valuable guide for primary care providers and their HIV positive female patients.

Pap smear Screening vs. Colposcopy in HIV:

Colposcopy involves direct examination of a woman's cervix with a special microscope and has several critical disadvantages. It requires management by a specialist and the procedure can be painful. Further, the biopsies that frequently accompany the colposcopic examination are invasive and entail a risk of bleeding or infection. Also this is not a cost effective way to identify cervical cancer in the general population.

Indications for Colposcopy:

- Cytologic abnormality (atypia or greater, including ASC, AGC)
- History of untreated abnormal Pap smear.
- periodic colposcopy after treatment of cervical dysplasia
- evidence of HPV infection
- CD4 <200/mm3

ASC-atypical squamous cells

AGC, atypical glandular cells

New Technologies under Investigation

These technologies are intended to reduce the false-negative rate, improve sensitivity and specificity of screening as well as the adequacy of the Pap smear thereby potentially improving laboratory productivity.

These new technologies include

- methods to improve the quality and adequacy of the Pap smear (liquid-based/thin-layer preparations)
- methods to improve Pap smear interpretation (computer-assisted screening)
- testing for the presence of high-risk human papilloma virus (HPV).

LIQUID-BASED/THIN-LAYER PREPARATIONS

(ThinPrep and Auto Cyte Preparation) [51,52]

The Pap smear is obtained with either a cytobrush and Ayre spatula or a cervical "broom" device. The clinician does not smear the sample directly onto a glass slide. Instead, the sample is placed in a small bottle containing fixative solution. The sample is sent to the cytology laboratory where it is filtered or centrifuged to remove excess blood and debris. The cells are then transferred to the slide in a "mono" layer. The slide is stained and examined manually in the conventional way.

Advantages of the liquid-based technique include improved transfer of cells from the collection device and uniformity of the cell population in each sample. This method provides representative residual material in collection media that can be used for additional adjunctive testing (e.g., HPV testing).

COMPUTER-ASSISTED SCREENING

(AutoPap and AutoCyte Screen) [53,54]

With **AutoPap**, the device reviews the material on the slide and, based on an algorithm, "scores" the slide as to the likelihood of an abnormality being present. This algorithm includes a variety of visual characteristics, such as shape and optical density of the cells. The device typically does not show the cytotechnologist which of the cells are likely to be abnormal.

The **AutoCyte** Screen device presents cell images to a human reviewer who then determines whether manual review is required. After the human reviewer has entered an opinion, the device reveals its determination based on a ranking as to whether manual review is warranted. When the human reviewer and the computer agree that no review is needed, a diagnosis of "within normal limits" is given. Manual review is required for any case if designated by either the cytologist or the computer ranking.

HPV TESTING [55,56,57]

Hybrid Capture II is the latest refinement of HPV tests and has been described as having enhanced sensitivity. It can detect 13 high-risk types of HPV. The sample is collected with a cervical swab from the transformation zone and placed into transport medium. The test may also be performed from residual material collected in liquid-based medium for monolayer preparation.

In the laboratory, cellular DNA is denatured and mixed with a ribonucleic acid probe that binds only to HPV DNA. The DNA "hybrid" is then captured by antibodies coating the sides of the tube. Next, a chemical is added, causing a chemoluminescent reaction. The amount of light that is measured can be used to determine the presence of HPV and the viral load.

However, the current evidence indicates that these new tests will increase the detection of lesions that may not be clinically meaningful. Specifically, most of the lesions detected are borderline or low-grade abnormalities. Unless new technology becomes cost effective, its application and utility will be limited.

Women who already have problems of access to the health care system are likely to face another burden that may further decrease their opportunity for screening. Providing better access to regular screening and consistent follow-up of patients with abnormal results is likely to have greater value than the implementation of new technology.

DISCUSSION

Invasive cervical cancer is an AIDS defining malignancy. Human uterine cervix is unique in that it is the site of an epithelial neoplasm occurring with high prevalence rate that is accessible, easily detectable, amenable to long term study with little discomfort and relative safety to patient.

The recognition of the cancer cervix in a truly early stage is one of the recent advances in the effort to control the disease.

Pap smear abnormalities in HIV infected women:

In the present study 45% of women had abnormal Pap smear, which included inflammation 14%, reactive atypia6%, various organisms 20% and epithelial abnormalities 5%.

This correlates with the study of Byrne et al (1989) who had reported abnormal Pap smears in 37% of HIV positive women.

Also it is similar to the observations made by Maiman et al (1998), who found that 30–60% of Pap smears exhibit cytological abnormalities and 15–40% have evidence of dysplasia.

The incidence of abnormal Pap smears for the HIV positive women in another study was 63%. (Provencher,1988)

TABLE 17: COMPARATIVE ANALYSIS OF PAP SMEAR ABNORMALITIES IN HIV INFECTED WOMEN.

S. No	Pap smear findings	Present Study	Byrne et al	Maiman et al	Provencher et al
1	Normal	55%	63%	40-70%	37%
2	Abnormal	45%	37%	30-60%	63%

Incidence of abnormal Pap smears was increased in HIV-infected women, and was associated with lower CD4 counts; progression and regression of Pap smear abnormalities had been associated with the level of immune suppression , as reflected in CD4 count (Massad , 2001; Schuman, 2003).

These results indicate that HIV infected women are more likely to have abnormal pap smears than HIV negative women.

HIV and gynecological problems:

In the present study 65% women had gynecological problems. Vaginal discharge was the most common presenting symptom (51%).

Minkoff et al (1999), had a similar observation in which 46.9% of 262 HIV infected women had at least one gynecological problems with serial assessment.

But this is less than the observations made by Frankel et al (1997) ,in which 83% of HIV positive women had gynecological problems when evaluated (Frankel, 1997).

These results indicate that gynecologic problems are common among HIV-positive women and are frequently present at the time of initial presentation for evaluation and care.

Age and Cervical cancer in HIV infected women:

In the present study, maximum incidence of epithelial abnormalities was between ages of 31-40 years. Both the patients with squamous cell carcinoma were under the age of 40 years.

This observation was similar to that of Lomalisa et al, (2000), who found that in HIV positive women cervical cancer presented between the ages of 35 and 40, which is 10-15 years earlier than expected in HIV negative women.

Malignancy in cervix is believed to begin to develop in adolescents or young adults and progress slowly over the decades. (Winkelstein, 1990; Feldman,1997).

In 1990, Maiman and colleagues evaluated 37 women diagnosed with invasive cervical cancer. Nineteen percent of the cancer patients under age 50 were HIV-positive.

These results showed that women with HIV and cervical cancer tend to be younger than HIV-negative women with cervical cancer.

CD4 count and epithelial abnormalities:

In the present study, maximum women with epithelial abnormalities had CD count between200-500. Two of them had SCC and one of them had LSIL. Mean CD4 count of women with epithelial abnormalities was 297.8 and mean CD4 count of women with SCC was 422.5.

This was similar to that of Lomalisa et al,(2000) who found that mean CD4 cell count was 443 cells/ mm3 at time of diagnosis of cervical cancer in women with HIV.

Both frequency and severity of abnormal Pap smears and histologically documented dysplasia increase with declining CD4 counts. (Garzetti, 1995; Shah, 1996; Davis, 2001).

But it was in contrast with the study of Mbulaiteye et al , (2003) who found that cervical cancer was not related to CD4 count .

HIV and HPV co infection in epithelial abnormalities:

In the present study one woman had HPV related LSIL, whose CD4 count was 131.

This observation was similar to the following studies. Women infected with both HIV and high risk HPV had a more than 40 fold higher risk of squamous intra epithelial lesions (SIL) than HIV non infected women (Moodely et al, 2006)

Sun et al. (Sun, 1995) have suggested that the presence of immune suppression shifts the ratio of latent: clinically expressed HPV infections from 8:1 in the general population to 3:1 in HIV-positive women with CD4 >500/mm3 to 1:1 in HIV-positive women with CD4 <200/mm3.

In HIV-positive women the prevalence and persistence of HPV infection increases with decreasing CD4 count and increasing HIV RNA levels (Palefsky,1999) and studies showed that oncogenic HPV types were more common with lower CD4 counts and/or higher viral loads(Luque 1999; Minkoff,1998). Higher HPV viral loads are also associated with lower CD4 counts (Heard, 2000).

Cross-sectional and longitudinal samples of HIV positive women demonstrated that both rates of HPV infection and disease and of high-grade dysplasia increase

as CD4 counts lower (Schafer, 1991; Vermund, 1991; Anastos, 1992; Wright, 1994)

These results indicate that oncogenic HPV types play a central role in the relationship between HIV and cervical cancer.

Incidence of various organisms and HIV:

In the present study 8% women had shift in flora (BV), 7% had candida and 4% had herpes virus. Maximum no of women with organisms had CD4 count less than 500.Mean CD4 count of shift in flora - 387.57, candida - 385, herpes virus-313.2.

In one comparative study 34% of HIV positive women had candidiasis, herpes - 11% and shift in flora 5 % (Archana Sharma, Y.S.Marfatia, Megha Modi -2009)

18–42% prevalence among HIV-infected women; BV is more prevalent and persistent as compared with HIV-negative controls and prevalence, persistence and severity increase with lower CD4 counts (Cu-Uvin, 1999; Greenblatt, 1999; Jamieson, 2001).

Studies suggest that BV or BV-associated organisms may enhance HIV transmission (Martin, 1999; Olinger, 1999).

BV has been associated with increased HIV expression in the genital tract (Cu-Uvin, 2001).

Analysis from the HER Study found that vulvovaginal candidiasis occurred with higher incidence and greater persistence, but not greater severity, among HIV-infected as compared to high-risk HIV-uninfected women.

Lower CD4 count was associated with vulvovaginal candidiasis (Cu-Uvin 1999;Duerr, 2003).

Incidence of Trichomoniasis in HIV-positive women 10–17% (Minkoff, 1999; Sorvillo, 1998).

Studies have not shown increased prevalence, incidence, persistence or recurrence compared with HIV-negative women or with lower CD4 counts (Cu-Uvin, 2002).

MATERIAL AND METHODS

This analytical study was carried out in the department of pathology, Coimbatore Medical College during the period of December 2008 to April 2010.Ethical clearance was obtained from institutution's ethics committee.

One Hundred HIV positive women attending STD/ Gynecology O.P were included in this study, after getting informed consent. Strict confidentiality was maintained throughout the study.

The age group varied from 20 years to 50years.A detailed history of the patient was obtained. General examination and pelvic examination were performed. Per speculum examination was performed to assess cervix. The patient was placed in lithotomy position. The Ayre's spatula was introduced through the external os and the squamo columnar junction was scraped by rotating the spatula to 360˚.The scraping was then evenly spread on to a glass slide which was immediately fixed using 95% isopropyl alcohol and proceeded with Papanicolaou staining. The slides were read and analyzed according to the Bethesda 2001 reporting guidelines.

Papanicolau's Staining Procedure: [58,59,60,61]

The three main advantages of this staining procedure are:

1) Good definition of nuclear detail.

2) Cytoplasmic transparency.

3) Indication of cellular differentiation of squamous epithelium.

Because of these qualities, the method has wide applications, especially in cancer and hormonal cytology.

It is a polychrome staining method which depends on degree of cellular maturity and cellular metabolic activity.

There are four main steps in the staining procedure:

1) Fixation.

2) Nuclear staining.

3) Cytoplasmic staining.

4) Clearing.

Reagents:

1) Harris' Hematoxylin:

2) OG 6:

Orange G - 0.5 (or) 1% solution in 95% alcohol	100ml
Phosphotungstic acid	0.015 gm

3) EA 36: (Eosin Alcohol)

Light green SF yellowish - 0.14% in 95% alcohol 45 ml

Bismarck brown Y-0.5% in 95% alcohol 10ml

Eosin Yellowish -0.55% in 95% alcohol 45 ml

Phosphotungstic acid 0.2 gm

Lithium carbonate, saturated aqueous solution 1drop

Fixation:

The cell samples are smears and must be well fixed in 95% isopropyl alcohol.

Nuclear staining:

The Hematoxylin nuclear stain is a natural stain which has been used for over 100 years .It has affinity for chromatin, attaching to sulphate groups on the D.N.A molecule.

Harris' Hematoxylin is commonly used in cytology and has the advantage of being usable almost immediately after preparation; where as other types need to mature. The regressive stain method gives especially clear contrast.

Cytoplasmic stain:

Eosin gives pink colour to:

(a) Cytoplasm of mature squamous cells.

(b) Nucleoli.

(c) Cilia.

Light green stains cytoplasm of metabolically active cells blue:

(a) Para basal squamous cells.

(b) Intermediate squamous cells.

(c) Columnar cells.

Bismarck brown does not add a characteristic colour to the cytoplasm.

Phosphotungstic acid has two functions in EA solution:

As an accentuator and mordant.

The effects of Orange-G are only evident in smear when keratinised cells are present. However it is likely that it enhances red blood cell staining and acts a mordant to EA stain.

Immerse cells in absolute alcohol, to dehydrate completely preparatory to clearing.

Clearing:

Clearing in Xylol results in cellular transparency and precedes mounting. Xylol is the commonest clearing agent and is miscible with both alcohol and D.P.X mountant.

Xylol is colourless , chemically nonreactive and has almost the same refractive index as glass which is important to give the best possible transparency of the image.

R.I .Xylol -1.494

R.I glass -1.51

Mounting:

The mountant

1. Acts as a permanent bond between slide and coverslip.
2. Protects cell material from air drying and shrinkage.
3. Acts as a seal against oxidation and fading of the stain

Observation and Results

A total of 100 HIV positive women were studied in the period between December 2008 to April 2010.

TABLE 1: SOCIO DEMOGRAPHIC CHARACTERS

S. No	Variables	No of cases n=100	%
1	Age in years		
	<=30 years	31	31%
	31-40 years	52	52%
	41-50 years	17	17%
2	Socio-economic status		
	Low	24	24%
	Middle	74	74%
	Higher	2	2%
3	Age at time of marriage		
	<20 years	52	52%
	20-30 years	46	46%
	>30 years	2	2%
4	Menopausal status		
	Pre menopausal	97	97%
	Post menopausal	3	3%
5	Literacy		
	Illiterate	28	28%
	$<5^{th}$ std	15	15%
	5^{th} -12^{th} std	53	53%
	Graduate	4	4%

In the present study, 52% of women were from the age group of 31-40 years. The youngest patient was 20 years and the oldest 50 years.

74% women belonged to middle socio economic status, 24% women were of lower economic status, where as only 2% women were of higher socio economic status.

52% of women were married at the age of 15-19 years. Mean age at marriage was 20.5 years. Only 2 women were married after 30 years.

TABLE 2: RESULTS OF PAP SMEAR

S. No	Variables	No of cases n=100	%
1	Negative for intra epithelial lesion or malignancy(NILM)	95	95%
	1.Noinflammation and epithelial abnormality	52	52%
	2. Inflammation	14	14%
	3.Reactive cellular changes	6	6%
	4 Organisms:		
	a. Shift in flora	8	8%
	b. Candida	7	7%
	c. Herpes simplex virus	4	4%
	d. Trichomonas vaginalis	1	1%
	5. Atrophic smears	3	3%
2	Epithelial abnormalities		
	LSIL	3	3%
	SCC	2	2%

55% of women had normal Pap smear and 45% had abnormal Pap smear. Inflammation was present in 14% of women. Reactive cellular changes were present in 6% women. Organisms were present in 20% women. LSIL was present in 3% of women and SCC was present in 2% women

TABLE 3: RELATIONSHIP BETWEEN AGE AND PAP SMEAR FINDINGS

S. No	Age in years	Pap Smear findings		
		Normal (n=55)	Abnormal (n=45)	Total (n=100)
1	<=30	16(51.6%)	15(48.4%)	31
2	31-40	27(51.9%)	25(48.1%)	52
3	41-50	12(70.6%)	5(29.4%)	17

Maximum incidence of abnormal Pap smear was between 31-40 years. Only 29.4% 0f women between 41-50 years had abnormal Pap smear.

Chi-Square Test

Value	df	Sig.
2.012(a)	2	.366

P value >0.05, indicates that the relationship between Age and Pap smear findings is statistically not significant. Hence it is concluded that Pap smear abnormalities could occur irrespective of age group.

TABLE 4: RELATIONSHIP BETWEEN PAP SMEAR AND SOCIO ECONOMIC STATUS

S. No	S.E status	Pap smear findings		
		Normal n=55	Abnormal n=45	Total n=100
1	Low	13(54.2%)	11(45.8%)	24
2	Middle	40(54.1%)	34(45.9%)	74
3	High	2(100%)	-	2

45.9% women from middle socio economic status had abnormal Pap smear. None of the women from high socio economic status had abnormal Pap smear.

Chi-Square Test

Value	df	Sig.
1.670	2	.434

P value <0.05; indicates that the relationship between Pap smear and socio economic status is statistically not significant. Hence it is concluded that Pap smear abnormalities could occur irrespective of socio economic status.

TABLE 5: COMPARISON OF AGE AND EPITHELIAL ABNORMALITIES

S. No	Age in years	Total no of women n=100	No of women with epithelial abnormalities		
			LSIL n=3	SCC n=2	Total n=5
1	<=30	31(31%)	-	-	-
2	31-40	52(52%)	2(3.85%)	2(3.85%)	4(7.70%)
3	41-50	17(17%)	1(5.88%)	-	1(5.88%)

Maximum incidence of epithelial abnormalities were between age of 31-40 years .Two of these had LSIL while the other two had SCC. Only one patient was between 41-50 years, who had LSIL.

TABLE 6:

COMPARISON OF SOCIO-ECONOMIC STATUS AND EPITHELIAL ABNORMALITIES

S. No	Socio economic Status	Total no of women n=100	No of women with epithelial abnormalities		
			LSIL n=3	SCC n=2	Total n=5
1	Low	24(24%)	2(8.33%)	-	2(8.33%)
2	Middle	74(74%)	1(1.35%)	2(2.70%)	3(4.05%)
3	Higher	2(2%)	-	-	-

All women with epithelial abnormalities were present in lower and middle socioeconomic group. None of them were in higher socio economic group.

TABLE 7:

COMPARISON OF AGE AT MARRIAGE AND EPITHELIAL ABNORMALITIES

S. No	Age at Marriage	Total no of Women	No of women with epithelial abnormalities		
			LSIL n=3	SCC n=2	Total n=5
1	<20	52(52%)	2(3.84%)	1(1.92%)	3(5.76%)
2	20-30	46(56%)	1(2.17%)	1(2.17%)	2(4.34%)
3	>30	2(2%)	-	-	-

Women with age at marriage <20 years only had maximum epithelial abnormalities. None of the women with epithelial abnormalities had age at marriage above 30 years.

TABLE 8:

COMPARISON OF LITERACY AND EPITHELIAL ABNORMALITIES

S. No	Literacy	Total no of Women n=100	No of women with epithelial abnormalities		
			LSIL n=3	SCC n=2	Total n=5
1	Illiterate	28(28%)	1(3.57%)	-	1(3.57%)
2	<5th std	15(15%)	-	2(13.33%)	2(13.33%)
3	5-12th std	53(53%)	2(3.77%)	-	2(3.77%)
4	Graduate	4(4%)	-	-	-

Epithelial abnormalities were present in women who studied <5thstd and up to 12thstd. Only 20% of them were illiterate .None of the graduates had epithelial abnormalities.

TABLE 9: DISTRIBUTION OF PRESENTING COMPLAINT

S.NO	Presenting complaint	No of Cases (n=100)	
		No	%
1	Discharge per vaginum	55	55%
2	Asymptomatic	35	35%
3	Urinary symptoms	3	3%
4	Itching	2	2%
5	Ulcers over genitalia	3	3%
6	Irregular bleeding	2	2%

65% of women had gynecological complaints and 35% of women were asymptomatic. Most common presenting complaint was discharge per vaginum (55%).

TABLE 10: RELATIONSHIP BETWEEN PRESENTING COMPLAINTS AND PAP SMEAR FINDINGS

S. No	Pap smear findings	Presenting complaints		
		Asymptomatic n=35	With complaints n=65	Total n=100
1	Normal	32(58.2%)	23(41.8%)	55
2	Abnormal	3(6.7%)	42(93.3%)	45

93.3% women with gynecological complaints had abnormal Pap smear. But only 6.7% women with no complaints had abnormal Pap smear.

Chi-Square test:

Value	Df	Sig
28.871	1	.000

P<0.01;indicates that the relationship between Presenting complaints and Pap smear findings is statistically significant.

TABLE 11: FINDINGS ON PER SPECULUM EXAMINATION:

Speculum findings	Cases	
	No	%
Normal cervix	49	49%
Erosion cervix	47	47%
Cervix bleed on touch	2	2%
Irregular growth cervix	1	1%
Venereal warts	1	1%

51% of women had abnormal cervix and 49% had normal cervix. Cervical erosion was present in 47% patients, cervix bleed on touch in 2% of women. One patient had an irregular growth on cervix.

TABLE 12: RELATIONSHIP BETWEEN PAP SMEAR RESULTS AND SPECULUM FINDINGS

S. No	Speculum findings	Pap smear results		
		Normal n=55	Abnormal n=45	Total
1	Normal Cervix	40(81.6%)	9(18.4%)	49
2	Abnormal Cervix	15(29.4%)	36(70.6%)	51

70.6% of women with abnormal cervix had abnormal Pap smear.

But only 18.4% women with normal cervix had abnormal Pap smear.

Chi-Square test:

Value	Df	Sig
25.465	1	.000

P value <0.01; indicates that the relationship between Pap smear results and speculum findings is statistically significant.

TABLE 13:

DISTRIBUTION OF WOMEN ACCORDING TO CD4 COUNT

S.No	CD4 count	Total no women n=100	
		No of cases	%
1	<200	32	32%
2	200-500	45	45%
3	500-700	14	14%
4	>700	9	9%

45% of women had CD4 count between200-500. 32% of women had CD4 count <200. Only 9% had CD4 count above 700.

TABLE 14:

RELATIONSHIP BETWEEN PAP SMEAR FINDINGS AND CD4 COUNT

S. No	CD4 count	Pap smear findings		
		Normal n=55	Abnormal n=45	Total n=100
1	<=200	17(53.1%)	15(46.9%)	32
2	201-500	23(51.1%)	22(48.9%)	45
3	501-700	7(50%)	7(50%)	14
4	>700	8(88.9)	1(11.1%)	9

48.9% of women with CD4 count between201-500 had abnormal Pap smear. Also 46.9% of women with CD4 count<=200 had abnormal Pap smear.

Chi-Square Test:

Value	df	Sig.
1.670	2	.434

P value >0.05; indicates that the relationship between Pap smear findings and CD4 count is not statistically significant. Hence it is concluded that Pap smear abnormalities could occur irrespective of age group.

TABLE 15:

RELATIONSHIP BETWEEN CD4 COUNT AND EPITHELIAL ABNORMALITIES

S. No	CD4 count	Total no of women n=100	No of women with epithelial abnormalities		
			LSIL n=3	SCC n=2	Total n=5
1	<200	32(32%)	2(6.25%)	-	2(6.25%)
2	200-500	45(45%)	1(2.22%)	2(4.44%)	3(6.66%)
3	500-700	14(14%)	-	-	-
4	>700	9(9%)	-	-	-

All women with epithelial abnormalities had CD count below 500. Two of them had SCC and one of them had LSIL. Mean CD4 count of women with epithelial abnormalities was 297.8 and mean CD4 count of women with SCC was 422.5.

TABLE 16:

COMPARISON OF VARIOUS ORGANISMS AND CD4 COUNT

S. No	CD4 count	Total no. of women	No of women with organisms				
			Candida n=7	HSV n=4	Tricho monas n=1	Shift in flora n=8	Total n=20
1	<200	32 (32%)	3 (9.38%)	1 (3.13%)	-	2 (6.25%)	6 (18.76%)
2	200-500	45 (45%)	2 (4.44%)	3 (6.66%)	-	3 (6.66%)	8 (17.76%)
3	500-700	14 (14%)	2 (14.29%)	-	1 (7.14%)	2 (14.29%)	5 (35.72%)
4	>700	9 (9%)	-	-	-	1 (11.11%)	1 (11.11%)

Maximum incidence of organisms had CD4 count less than 500.

Mean CD4 count of shift in flora - 387.57, candida - 385, herpes

Virus -313.

SUMMARY

- The current study was conducted on 100 HIV infected women, attending Coimbatore medical college Hospital.
- In the present study 65% women had gynecological problems. Vaginal discharge was the most common presenting symptom (51%).
- Incidence of abnormal Pap smears was increased in HIV infected women.
- In the present study 45% of women had abnormal Pap smear, which includes inflammation 14%, reactive atypia 6%, various organisms 20% and epithelial abnormalities 5%.
- In the present study 8% women had shift in flora, 7% had candida and 4% had herpes virus. Maximum no of women with organisms had CD4 count less than 500.Mean CD4 count of shift in flora - 387.57, candida - 385, herpes virus-313.2.
- Women with HIV and cervical cancer tend to be younger than HIV negative women with cervical cancer.
- In the present study, maximum incidence of epithelial abnormalities were between age of 31-40 years . Both the cases of squamous cell carcinoma were under the age of 40 years.

- Women infected with both HIV and high risk HPV had high risk of squamous intra epithelial lesions (SIL) than HIV non infected women.
- In the present study one women had HPV related LSIL, whose CD4 count is 131.
- In the present study, maximum women with epithelial abnormalities had CD4 count between 200-500.Two of them had squamous cell carcinoma (SCC) and one of them had Low grade intra epithelial lesion (LSIL).
- Mean CD4 count of women with epithelial abnormalities was 297.8 and mean CD4 count of women with SCC was 422.5

CONCLUSION

- Reproductive tract morbidities are common in HIV infected women. Hence, all HIV positive women must be screened periodically by Pap smear for evidence of genital tract involvement, so that they can be promptly and effectively treated.
- Incidence of abnormal Pap smears is higher in HIV infected women. Exfoliative cervico vaginal cytology has been regarded as the gold standard for cervical screening programs. Pap smear is widely recognized as the most cost effective cancer screening test devised and serves as a model for screening of other malignancies.
- The availability of cervical cytology to screen for cervical cancer often permits diagnosis at the pre invasive stage, when treatment can almost always prevent progression to invasive cancer.
- For this reason, screening for cervical cancer is important in HIV infected women since the incidence of cervical intraepithelial neoplasia and cancer cervix are four to five times higher in HIV-positive women.

- Also women with HIV and cervical cancer tend to be younger than HIV negative women with cervical cancer.
- Hence our study conclude that all HIV positive women should be regularly followed with Pap smear for evidence of lower genital tract neoplasia, regardless of age, CD4 count or viral load.

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MASTER CHART

S.NO	ART NO	AGE IN YRS	AGE AT MARRIAGE	CD4 COUNT	SPOUSE HIV STATUS	EXTRA MARITAL CONTACT	S.E STATUS	LITERACY	DURATION OF ART	LMP	LCB	PRESENTING COMPLAINT	P/V&SPECULUM FINDINGS	PAP SMEAR REPORT
1	A0079	37	23	253	+	+	Middle	2nd std	3yrs	7th day	6 yrs	No complaints	NAD	NILM
2	A0200	28	25	234	+	_	Middle	8th std	3.5 yrs	20th day	2 Yrs	No complaints	Cervix NAD	NILM
3	A0216	45	23	578	+	_	Low	8th std	3 yrs	7th day	12 Yrs	Mild white Discharge	Cervix NAD	NILM
4	A0252	43	25	560	+	_	Middle	5th std	3.5 yrs	15th day	20 Yrs	Mild white Discharge	Erosion Cervix	NILM/Shift in flora
5	A0308	33	28	472	+	_	Middle	B.A	3.5 yrs	20thday	8 yrs	White Discharge	Erosion both cervical lips	NILM / Reactive cellular changes
6	A0412	43	20	32	+	+	Middle	_	4 yrs	20thday	17 yrs	_	Erosion cervix	NILM

7	A0419	29	23	180	+	_	Middle	10th std	3.5 yrs	7th day	9yrs	Dysuria	Mild erosion cervix	NILM
8	A0482	37	24	227	+	_	High	B Com	3 yrs	25th day	12 yrs	_	NAD	NILM
9	A0512	28	21	224	+	_	Middle	10th std	3.5 yrs	22nd day	4yrs	_	NAD	NILM
10	A0533	40	16	310	+	+	Middle	8th std	3yrs	10th day	19 yrs	_	NAD	NILM
11	A0762	36	27	846	+	_	Middle	7th std	2.5 yrs	10th day	7 yrs	-	NAD	NILM
12	A0800	37	24	227	+	_	High	BCom	2.5 yrs	25th day	12 yrs	_	NAD	NILM
13	A0828	35	20	179	_(2nd marriage)	+	Middle	3rd std	2.5 yrs	irregular	_	Itching	NAD	NILM/ Reactive Cellular changes
14	A0959	50	18	909	+	+	Low	8th std	2 yrs	Post menopausal	23 yrs	Curdy discharge	CX NAD	NILM/ Atrophic smear
15	A0979	42	30	529	+	+	Middle	_	2.5 yrs	irregular	14 yrs	Mild white discharge P/V	CX NAD	NILM
16	A0989	35	23	1056	+	_	Middle	12th std	3 yrs	10th day	11yrs	_	NAD	NILM
17	A1007	47	19	31	+	_	Low	_	2.5 yrs	15th day	23 yrs	_	NAD	NILM

18	A1014	36	20	1059	+	_	Middle	_	1 yr	18th day	_	Dysuria	NAD	NILM
19	A1198	35	15	280	+	_	Middle	5th std	2.5 yrs	8th day	14 yrs	Thick white discharge	Erosion CX	NLIM
20	A1213	33	15	456	+	_	Low	_	3 yrs	2 wks	13 yrs	white discharge	Erosion CX	NILM/Inflammation
21	A1452	37	18	500	+	_	Middle	_	2.5 yrs	irregular	_	-	NAD	NILM
22	A1491	37	19	132	+	_	Middle	_	2 yrs	7th day	21 yrs	_	NAD	NILM
23	A1524	28	19	194	+	_	Middle	5th std	2 yrs	20th day	3 yrs	curdy white discharge	Erosion CX	NILM/ Candida
24	A1555	29	19	167	+	+	Middle	5th std	1.5 yrs	7th day	8 yrs	Thin white discharge	NAD	NILM
25	A1630	40	18	572	+	+	Middle	_	1.5 yrs	15th day	20 yrs	Itching	Erosion CX	NILM
26	A1736	30	20	311	_	+	Middle	6th std	2 yrs	20th day	7 yrs	Thin white discharge	Erosion CX	NILM
27	A1737	35	19	273	+	+	Low	2nd std	1.5 yrs	20th day	14 yrs	Thick curdy white discharge	Erosion CX	NILM/Candida
28	A1739	40	17	256	+	+	Low	_	2.5 yrs	10th day	23 yrs	_	NAD	NILM
29	A1746	31	24	165	+	_	Middle	12th std	2 yrs	17th day	6 yrs	-	NAD	NILM
30	A1791	48	17	247	+	_	Middle	_	2.5 yrs	16th day	19 yrs	Mild white discharge	Erosion CX	NILM

31	A1797	36	25	413	+	_	Middle	3rd std	1.5 yrs	12th day	9 yrs	_	NAD	NILM
32	A1834	39	19	21	_	+	Low	5th std	1.5 yrs	6th day	16 yrs	_	NAD	NILM
33	A1880	25	20	296	+	_	Middle	10th std	6 months	25th day	4 yrs	_	NAD	NILM
34	A1956	46	32	364	_	+	Low	_	1.5 yrs	Irregular	12 yrs	Itching	Erosion CX	NILM
35	A1976	29	19	64	+	_	Middle	10th std	9 months	20th day	7 yrs	Mild white discharge	NAD	NILM
36	A2030	32	20	114	+	+	Middle	-	1.5yrs	irregular	_	_	Erosion CX	NILM/ Inflammation
37	A2139	35	17	648	+	_	Middle	3rd std	1.5 yrs	15th day	14 yrs	_	NAD	NILM
38	A2209	37	20	277	+	_	Middle	_	7months	10th day	13 yrs	white discharge	NAD	NILM
39	A2343	40	18	229	_	+	Middle	_	1.5 yrs	20th day	18 yrs	Mild white discharge P/V	Erosion CX	NILM/ Reactive cellular changes
40	A2388	35	25	176	+	_	Middle	9th std	2 yrs	2 weeks	10 yrs	white discharge P/V	Erosion CX	NILM/ Reactive cellular changes
41	A2441	30	21	119	+	_	Low	12th std	1 yr	15th day	6 yrs	Mild white discharge P/V	Erosion CX	NILM/Inflammation
42	A2466	50	23	83	+	+	Low	_	1 yr	Menopause	20 yrs	_	NAD	Atrophic smear/ NILM
43	A2487	27	20	70	+	_	Middle	_	1 yr	15th day	_	white discharge	NAD	NILM/shift in flora
44	A2568	35	15	163	+	+	Middle	_	1 yr	15th day	4yrs	_	_	NILM

45	A2653	33	27	203	+	_	Low	8th std	10months	25th day	2 yrs	white discharge	Erosion CX	NILM/shift in flora
46	A2684	40	35	480	+	+	Low	-	10months	25th day	7 yrs	white discharge P/V	Erosion CX	NILM/shift in flora
47	A2743	29	27	161	+	_	Middle	12th std	10 months	15th day	7months	-	NAD	NILM
48	A2747	35	21	194	_	+	Middle	2nd std	5months	15th day	6 yrs	white discharge P/V	Erosion CX	NILM/ Candida
49	A2835	42	20	131	+	_	Low	_	8months	Irregular	9 yrs	White discharge P/V	Multiple Venereal warts	LSIL/ HPV related
50	A2897	42	19	132	_	+	Middle	_	10 months	10th day	25 yrs	-	NAD	NILM/ shift in flora
51	A2919	48	20	187	+	_	Low	_	1.5 yrs	menopause	_	_	CX- NAD	NLIM/ Atrophic smear
52	A2977	32	15	215	+	+	Middle	_	6months	7th day	15 yrs	_	NAD	NILM/shift in flora
53	A3023	22	19	35	+	+	Middle	10th std	6months	Irregular	1yr	Dysuria	NAD	NILM/Inflammation
54	A3030	31	16	191	+	_	Low	4th std	6months	8th day	5 yrs	_	NAD	NILM
55	A3034	37	18	54	+	_	Middle	10th std	1 year	18th day	7 yrs	_	Erosion Cx	NILM/candida
56	A3046	42	18	989	+	_	Middle	-	6months	8th day	15 yrs	mild white discharge P/V	Erosion Cx	NILM/shift in flora

57	A3083	30	17	237	+	_	Low	8th std	5months	10th day	8 yrs	mild white discharge P/V	Erosion Cx	NILM
58	A3149	44	15	151	+	_	Middle	_	4months	7th day	20 yrs	mild white discharge P/V	Erosion Cx	NILM
59	A3164	28	17	272	+	_	low	9th std	6months	25th day	9 yrs	mild white discharge P/V	Erosion Cx	NILM/ Inflammation
60	A3200	35	24	175	+	_	Middle	_	4months	7th day	12 yrs	mild white discharge P/V	Erosion Cx	NILM
61	A3220	34	17	254	_	+	Middle (CSW)	8th Std	4months	10th Day	10 yrs	Multiple small ulcers-vulva	Erosion Cx	NILM/Herpes simplex virus
62	A3246	38	22	259	+	+	Middle	8th std	2months	11th Day	10 Yrs	mild white discharge P/V	Erosion Cx	NILM/ Inflammation
63	A3256	28	17	251	+	_	Middle	3rd std	2months	20th Day	13 Yrs	_	NAD	NILM
64	A3271	30	18	26	+	_	Middle	4th std	2months	irregular	9 Yrs	mild white discharge P/V	Erosion Cx	NILM/ Reactive cellular changes
65	A3312	22	19	333	+	+	Low	7th std	1 month	4th day	10Yrs	Profuse foul smelling discharge P/V	Erosion CX	NILM/ Inflammation
66	A3315	35	16	224	+	_	Low	_	1 month	17th Day	12 yrs	_	NAD	NILM
67	A3346	34	19	265	+	_	Middle	8th std	1 month	8th day	_	mild white discharge P/V	Erosion Cx Cu-T insitu	NILM/ Inflammation
68	A3392	28	23	94	+	_	Middle	BSC	15 days	irregular	4 Yrs	Ulcers over labia.	Erosion Cx	NILM/Herpes simplex virus

69	A3404	37	18	431	-	+	Middle	5th std	15 days	20th Day	18 Yrs	Mild white discharge P/V	Erosion Cx	LSIL
70	S0344	39	18	344	+	_	Middle	5th std	_	20th Day	12 Yrs	Mild white discharge P/V	Erosion Cx	NILM
71	S1041	24	19	412	+	_	Middle	10th std	_	20th Day	1.5 yrs	-	-	NILM
72	S1066	31	23	827	+	_	Middle	8th std	_	10th day	7 Yrs	white discharge	Erosion Cx	NILM
73	S1076	23	19	313	+	+	Middle	8th Std	_	25th Day	5 Yrs	Vesicles over genitalia	Erosion Cx	NILM/Herpes simplex virus
74	S1256	22	20	792	+	_	Middle	6th std	_	25th Day	2 Yrs	white discharge	Erosion Cx	NILM
75	S1752	31	21	315	+	+	Middle	10th std	_	20th Day	10 Yrs	Curdy white discharge P/V	Erosion Cx	NILM/ Inflammation
76	S2466	38	19	576	+	_	Middle	5th std	_	12th Day	8Yrs	Greenish discharge P/V	Erosion Cx	NILM/ Trichomonas vaginalis
77	S2606	30	25	630	+	_	Middle	12th std	_	12th Day	8months	white discharge P/V	Erosion Cx	NILM
78	S2698	20	17	592	+	_	Middle	10th std	_	25th Day	_	Ulcers over genitalia	Erosion Cx	NILM/Herpes simplex virus
79	S2913	37	19	277	+	+	Middle	5th std	_	14th Day	13 Yrs	_	NAD	NILM
80	S3653	23	17	272	+	+2nd marriage	Middle	10th std	_	25th Day	7 Yrs	white discharge P/V	Erosion Cx	NILM/Reactive cellular changes

81	S4897	45	19	838	+	+	Middle	_	_	20th Day	24 Yrs	white discharge	Erosion Cx	NILM
82	S4395	28	22	544	+	_	Middle	12th std	_	2 weeks	5 Yrs	white discharge P/V	NAD	NILM/ Shift in flora
83	S5183	24	21	679	+	_	Middle	10th std	_	7thday	1 Yr	white discharge P/V	Erosion, curdy white discharge	NILM/Candida
84	S5268	26	18	529	+	_	Middle	8th std	_	7th Day	10 Yr	white discharge P/V	Erosion Cx	NILM
85	S5702	43	19	628	+	+	Low	2nd Std	_	10th day	15 Yrs	white discharge P/V	Erosion Cx	NILM/Candida
86	S5728	31	22	185	+	_	Middle	12th std	_	7th Day	8 yrs	-	-	NILM
87	S5830	35	19	396	+	_	Middle	2nd std	_	irregular	10 Yrs	Irregular bleeding P/V	Ulcerative growth CX, bleed on touch	SCC
88	S5873	35	15	472	+	_	Middle	4th std	_	10th Day	10 Yrs	white discharge	Erosion Cx	NILM/ Inflammation
89	S6043	39	23	449	+	_	Middle	3rd std	_	irregular	10 Yrs	excessive white discharge P/V	Bleed on touch	SCC
90	S6150	27	21	499	+	_	Middle	9th std	_	10th day	9 Yrs	white discharge P/V	NAD	NILM/ Inflammation
91	S6180	33	20	342	+	+	Middle	8th std	_	7th Day	2 Yrs	White discharge P/V	NAD	NILM/Candida
92	S6413	30	27	432	+	_	Middle	8th std	_	2 weeks	2 Yrs	_	NAD	NILM
93	S6904	27	22	562	+	_	Middle	5th std	_	ANC-5MA	_	White discharge P/V	NAD	NILM/ Inflammation

94	S6905	35	21	468	+	_	Middle	5th std	_	14th Day	10 Yrs	White discharge P/V	NAD	NILM/ Inflammation
95	S6933	36	19	202	+	_	Middle	2nd std	_	25th Day	14 yrs	-	NAD	NILM
96	S6977	29	17	565	+	_	low	8th std	_	20th Day	2.5 Yrs	-	NAD	NILM
97	S6994	40	25	122	+	+	low	_	_	irregular	_	_	NAD	NILM
98	S7052	45	19	768	+	_	Middle	2nd std	-	14th day	12 yrs	_	NAD	NILM
99	S7061	32	18	82	+	+	low	5th std	_	14th day	10 Yrs	Excessive white discharge	Erosion CX	LSIL
100	S7082	34	19	120	+	_	low	8th std		10th day	11 yrs	Mild white discharge P/V	Erosion CX	NILM/ Inflammation

LMP-Last menstrual period

LCB-Last child birth

NAD – No abnormality detected

S.E status- Socio economic status

NILM - Negative for Intra epithelial lesion or Malignancy

LSIL- Low grade squamous intraepithelial lesion

SCC - Squamous cell carcinoma

PAPANICOLAOU STAINING PROCEDURE

1. Slides were transferred directly from fixative, without drying, to 80% alcohol and bring down through 70 and 50% alcohols to water.
2. Stained in Harris' hematoxylin for 4 minutes.
3. Rinsed briefly in water. (All rinsing was gentle)
4. Dipped in 0.25% HCl in alcohol about six times.
5. Placed in running tape water for 6 minutes.
6. Rinsed in distilled water & run through 50%, 70%, 80% to 95% alcohol.
7. Stained in OG 6 for 2 to 4 minutes.
8. Rinsed in two changes of 95% alcohol.
9. Stained in EA 36 for 2 to 4 minutes.
10. Rinsed in three changes of 95% alcohol.
11. Dehydrated in absolute alcohol, followed by equal parts of absolute alcohol and xylol, cleared in xylol and mounted.

PROFORMA

1) Name

2) Age

3) Sex

4) IP\OP NO

5) HIV status

6) Education

7) Occupation

8) Age at menarche

9) Age at marriage

10) Menstrual history

11) Sexual history, H/O extra marital contact

12) Parity

13) Last child birth

14) Excessive discharge Per Vaginum

(Nature, colour , odour and consistency)

15) Bleeding Per Vaginum

16) Post coital bleeding

17) Pain abdomen, low back ache

18) Bladder and Bowel habits

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